

1. (Original) A substantially pure human or rabbit 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase  $B\alpha$ , wherein the protein kinase comprises the peptide sequence as shown in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5.

2. (Original) A substantially pure human or rabbit 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase  $B\alpha$ , wherein the protein kinase has a molecular weight of about 67 kDa as determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis, and wherein the 3-phosphoinositide-dependent protein kinase comprises the peptide sequence as shown in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5.

3. (Original) A substantially pure 3-phosphoinositide dependent protein kinase having the amino acid sequence as shown in SEQ ID. NO:1 with from 1 to 4 conservative substitutions therein.

4. (Original) A substantially pure human or rabbit 3-phosphoinositide-dependent protein kinase according to claim 1 that phosphorylates and activates protein kinase  $B\alpha$ .

5. (Original) A substantially pure human or rabbit phosphatidylinositol-3,4,5-triphosphate-dependent protein kinase according to Claim 1.

6. (Original) A protein kinase according to Claim 1 which activates protein kinase  $B\alpha$  in the presence of the D-enantiomer of *sn*-1-stearoyl-2-arachidonyl phosphatidylinositol 3,4,5-trisphosphate but does not activate protein kinase  $B\alpha$  in the presence of the L-enantiomer of the said phosphatidylinositol 3,4,5-trisphosphate.

7. (Original) A protein kinase according to Claim 1 which is activated by the D-enantiomer of *sn*-1,2-dipalmitoyl phosphatidylinositol 3,4,5-trisphosphate or *sn*-1,2-dipalmitoyl phosphatidylinositol 3,4-bisphosphate but is not activated by the L-enantiomers of the said phosphatidyl inositol phosphates.

8. (Original) A protein kinase according to Claim 1 which is not activated by phosphatidylinositol 3,5-bisphosphate or phosphatidylinositol 4,5-bisphosphate or phosphatidylinositol 4-phosphate or phosphatidylinositol 3-phosphate or inositol 1,3,4,5-tetrakisphosphate.

9. (Original) A protein kinase according to claim 1 isolated from rabbit skeletal muscle.

10. (Original) A polynucleotide encoding the protein kinase of claim 1.

11. (Original) A polynucleotide according to claim 10 which contains no introns.

12. (Original) A vector comprising the polynucleotide of claim 10.

13. (Original) A host cell comprising the polynucleotide of claim 10.

14. (Original) A host cell comprising the vector of claim 12.

15. (Original) A method of making a 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase B $\alpha$  or a fragment or fusion of said protein

kinase, the method comprising culturing a host cell of claim 13 which expresses said 3-phosphoinositide-dependent protein kinase and isolating said 3-phosphoinositide-dependent protein kinase from said host culture.

16. (Original) A method of isolating a protein kinase according to claim 1, the method comprising the steps of (a) obtaining material that contains said protein kinase, (b) obtaining cell free extracts from said material which contain said protein kinase, (c) fractionating said cell free extract and (d) selecting a fraction from step (c) which is capable of phosphorylating and activating protein kinase B $\alpha$  in the presence of a 3-phosphoinositide.

17. (Original) A human or rabbit 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase B $\alpha$  or a fusion protein comprising said protein kinase or a fragment of said fusion protein or a fusion protein comprising said fragment obtainable by the method of Claim 16.

18. (Original) A method of identifying a compound that modulates the activity of a protein kinase according to claim 1, the method comprising:

contacting a compound with said protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment and

determining whether, in the presence of said compound, phosphorylation and activation of a protein kinase B or phosphorylation of a p70 S6 kinase is changed compared to the phosphorylation and activation of a protein kinase B or phosphorylation of a p70 S6 kinase in the absence of said

compound.

19. (Currently Amended) A method according to Claim ~~20~~  
18 wherein the compound decreases the activity of the 3-  
phosphoinositide-dependent protein kinase.

20. (Currently Amended) A method according to Claim ~~20~~  
18 wherein the compound increases the activity of the 3-  
phosphoinositide-dependent protein kinase.

21. (Currently Amended) A method according to Claim ~~20~~  
18 wherein the compound competes with a 3-phosphoinositide.

22. (Currently Amended) A method according to Claim ~~23~~  
21 wherein the compound prevents activation of protein kinase  
B $\alpha$  in the presence of phosphatidylinositol-3,4,5-trisphosphate  
or phosphatidylinositol-3,4-bisphosphate.

23. (Currently Amended) A method according to Claim ~~20~~  
18 wherein the compound modulates the activity of a human or  
rabbit 3-phosphoinositide-dependent protein kinase by binding  
to the protein kinase B $\alpha$  and preventing phosphorylation and  
activation of protein kinase B $\alpha$  by the 3-phosphoinositide-  
dependent protein kinase.

24. (Original) A method of identifying a compound that  
mimics the effect of a 3-phosphoinositide on a protein kinase  
according to claim 1, the method comprising determining  
whether said compound activates the protein kinase according  
to claim 1 or a fusion protein comprising said protein kinase  
according to claim 1 or a fragment of said fusion protein  
having 3-phosphoinositide-dependent protein kinase activity or  
a fusion protein comprising said fragment so that it can  
phosphorylate and activate a protein kinase B or phosphorylate

a p70 S6 kinase, the activation by said compound being in the absence of a phosphatidylinositol-3,4,5-triphosphate or a phosphatidyl-inositol-3,4-bisphosphate or another 3-phosphoinositide.

25. (Currently Amended) A method according to Claim ~~26~~ 24 wherein the 3-phosphoinositide is phosphatidylinositol-3,4,5-trisphosphate or phosphatidyl-inositol-3,4-bisphosphate.

26. (Original) A method of screening for compounds which modulate the activity of a protein kinase according to claim 1, or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment, or compounds which modulate their interactions with a 3-phosphoinositide or with protein kinase B, wherein said screening comprises:

contacting a compound with the protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment and

selecting compounds which modulate the activity of said protein kinase according to claim 1 or a fusion protein comprising said protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment.

27. (Original) A method of activating protein kinase B the method comprising contacting said protein kinase B with a 3-phosphoinositide-dependent protein kinase according to Claim 1.

28. (Currently Amended) A kit comprising substantially pure human or rabbit 3-phosphoinositide-dependent protein kinase that phosphorylates and activates protein kinase B $\alpha$ , wherein the protein kinase comprises the peptide sequence as shown in SEQ ID NO:3, SEQ ID NO:4, or SEQ ID NO:5 or a fusion protein comprising said protein kinase or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment and a means for carrying out the method according to Claim ~~20~~ 18.

29. (Original) A method of activating protein kinase B comprising contacting the protein kinase B with a 3-phosphoinositide-dependent protein kinase as defined in claim 1.

30. (Original) A fusion protein comprising the protein kinase according to claim 1 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment.

31. (Original) A substantially pure 3-phosphoinositide-dependent protein kinase wherein the protein kinase comprises amino acids 52-556 of SEQ ID NO:1 or amino acids 52-556 of SEQ ID NO:1 with from 1 to 4 conservative substitutions therein.

32. (Currently Amended) A fusion protein comprising the protein kinase according to claim ~~33~~ 31 or a fragment of said fusion protein having 3-phosphoinositide-dependent protein kinase activity or a fusion protein comprising said fragment.

33. (Currently Amended) A ~~polypeptide~~ polynucleotide encoding the protein kinase of claim ~~32~~ 30.

34. (Currently Amended) A ~~polypeptide~~ polynucleotide encoding the protein kinase of claim ~~33~~ 31.

35. (Currently Amended) A ~~polypeptide~~ polynucleotide encoding the protein kinase of claim ~~34~~ 32.